UNIVERSITI PUTRA MALAYSIA

MICROBIOLOGICAL AND BIOCHEMICAL CHANGES OF FRESHWATER PRAWNS (*MACROBRACHIUM ROSENBERGII*) DURING STORAGE

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By

FATIMAH BINTI ABU BAKAR

Thesis Submitted in Fulfilment of the Requirement for the Degree of Doctor of Philosophy in the Faculty of Science and Environmental Studies Universiti Putra Malaysia

March 2001

Lovingly for Abang,

Ayong and O'meil,

They matter more, above all else……

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy.

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March 2001

Chairman: **Associate Professor Dr. Che Nyonya Abdul Razak**

Faculty: **Science and Environmental Studies**

Tropical freshwater prawns are of considerable interest because of the importance of this commodity in international trade. They are one of the highest valued products in international fisheries trade and as such have good long term potential. Studies on the bacteriological and biochemical changes of pond water and cultured freshwater prawns were carried out to evaluate the relationship between water quality and microbiological changes during storage. Prawns were taken from three sampling sites viz: Site 1- Kg. Jumbang, Negri Sembilan; Site 2- Kg. Cangkat Tin, Perak and Site 3- Kg. Cenderiang, Perak. They were then stored at 3 different storage conditions: 1) 20 d at ambient (28° ± 2°C); 2) 10 d at 10° ± 2°C and 3) 16 d at iced storage (4° ± 2°C). Microbiological analysis was performed for total mesophilic and psychrophilic aerobic counts, proteolytic bacterial counts, histamine producing bacteria, cadaverine producing bacteria and putrescine producing bacteria in the prawns and pond water for the three sites using appropriate

bacteriological agar media. Biochemical analysis was carried out for pH profiles using pH meter, freshness in terms of K-values from the test paper strips, amino acids profiles using the amino acid analyser, total volatile bases (TVB) and total volatile acids (TVA) from the distillation methods and biogenic amines viz; histamine, cadaverine and putrescine using the high performance liquid chromatography (HPLC) method. Results obtained showed that the microbiological quality of freshwater prawns was related to the microflora of pond water where they were grown. The initial counts indicated the values were in the range of log 4+ CFU/g for all samples. Total mesophilic and psychrophilic counts of the head regions were higher than that of the body regions for all prawn samples and types of growth media tested. All samples showed an increase in counts with time and temperature of storage up to log 7+ CFU/g for mesophilic counts after 12 h at ambient, 6 d at 10°C and 12 d at iced storage. Site 2 samples had relatively higher counts as compared to that of the other two sites which correlated well with the levels determined in their pond water. Similar trends were observed for psychrophilic counts but at lower values for different types of media studied. Biochemical results showed similar trends of increment in values and were closely related to the microbiological data until spoilage set in.

Effect of preservatives on quality changes, biogenic amines production and shelf life of prawns during iced storage were investigated by sensory, microbiological, physical and chemical analyses. Sensory results indicated that the whole prawns had a shelf life of about 20 d when treated with 2% sucrose, boric acid, lactic acid, sodium chloride, and sodium metabisulfite except for control

which had only 15 d shelf life. Boric acid, lactic acid and sodium metabisulfite managed to inhibit psychrophilic bacteria and biogenic amines formation in prawns while maintaining the mesophilic counts at lower levels during iced storage.

Different analytical methods for the determination of histamine in prawns were evaluated. Use of enzymatic methods in the form of oxygen electrode and spectrophotometer offered excellent alternative methods for determination of histamine in freshwater prawns. The experimental results showed there was an increase of histamine measured by the oxygen electrode and very good correlation of above 0.98 with the HPLC analyser for all samples. Recovery percentages of about 100% for values of histamine between 0-200 μg/g was observed. Similarly, use of spectrophotometer with coupled diamine oxidase and horseradish peroxidase in the system allowed a semi quantitative estimation of histamine in prawns. Correlation coefficients of $r^2 = 0.99$ were observed when compared to that of the HPLC method. Percentages of recovery of histamine in augmented samples were also about 100% for concentration of histamine between 0-200 μg/g.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

PERUBAHAN MIKROBIOLOGI DAN BIOKIMIA UDANG AIR TAWAR *(MACROBRACHIUM ROSENBERGII)* SEMASA PENYIMPANAN

Oleh

FATIMAH BINTI ABU BAKAR

Mac 2001

Pengerusi: **Profesor Madya Che Nyonya Abdul Razak, Ph.D.**

Fakulti: **Sains dan Pengajian Alam Sekitar**

Minat yang mendalam terhadap udang air tawar tropika menyebabkan komoditi ini penting dalam perdagangan antarabangsa. Ia merupakan satu daripada produk bermutu tertinggi dalam pasaran perikanan antarabangsa dan justeru itu mempunyai potensi jangka panjang yang baik. Kajian tentang perubahan bakteriologi dan biokimia ke atas air kolam dan udang air tawar peliharaan telah dijalankan daripada tiga tapak persampelan iaitu: Tapak 1 – Kg. Jumbang, Negri Sembilan; Tapak 2 – Kg. Cangkat Tin, Perak dan Tapak 3 – Kg. Cenderiang, Perak. Sampel udang hidup daripada tiga tapak berbeza dibawa balik ke makmal dalam beg polietina beroksigen sebelum disimpan selama 20 j pada suhu bilik (28° ± 2°C), 10 h pada 10° ± 2°C dan 16 h pada simpanan berais (4° ± 2° C). Analisis mikrobiologi dijalankan bagi jumlah kiraan aerob mesofilik dan psikrofilik, kiraan bakteria proteolitik, bakteria penghasil histamina, bakteria penghasil kadaverina dan penghasil putresina dalam udang dan air kolam untuk ketiga-tiga tapak dengan menggunakan medium agar bakteriologi yang

sesuai. Analisis biokimia telah dijalankan bagi profil pH menggunakan meter pH, kesegaran daripada aspek nilai- K menggunakan strip kertas ujian, profil asid amino menggunakan penganilisis asid amino, jumlah bes meruwap dan jumlah asid meruap melalui kaedah penyulingan dan amin biogenik seperti; histamina, kadaverina dan putresina menggunakan kaedah kromatografi cecair berprestasi tinggi (HPLC). Keputusan diperolehi menunjukkan mutu mikrobiologi udang air tawar adalah berhubungkait dengan mikroflora air kolam di mana udang membiak. Kiraan awal menunjukkan nilai bakteria adalah dalam julat log 4+ CFU/g bagi kesemua sampel. Jumlah kiraan aerob mesofilik dan psikrofilik terdapat pada bahagian kepala adalah lebih tinggi berbanding dengan bahagian badan udang bagi kesemua sampel dan jenis medium pertumbuhan yang diuji. Semua sampel mempamerkan peningkatan dalam bilangan mengikut masa dan suhu penstoran sehingga mencapai log 7+ CFU/g bagi mengikut kiraan mesofilik selepas 12 j pada suhu bilik, 6 h pada 10°C dan 12 h pada penyimpanan ais. Sampel dari Tapak 2 mempunyai bilangan bakteria yang lebih tinggi berbanding dengan dua tapak yang lain, di mana ini berkait rapat dengan bilangan yang diperolehi dalam air kolam masing-masing. Pola yang sama dilihat bagi kiraan psikrofilik tetapi pada nilai yang lebih rendah bagi jenis medium berbeza yang dikaji. Keputusan ujian biokimia juga menunjukkan corak peningkatan yang sama dalam nilai dan berkait rapat dengan data mikrobiologi sehingga mencapai tahap kerosakan.

Kesan pengawet ke atas perubahan mutu dan hayat simpan udang semasa penyimpanan berais telah dikaji melalui analisis penilaian deria, mikrobiologi, fizikal dan kimia. Keputusan penilaian deria menunjukkan bahawa udang mempunyai hayat

simpan selama lebih kurang 20 h apabila dirawat dengan 2% sukrosa, asid borik, asid laktik, sodium klorida, dan sodium metabisulfit kecuali sampel kawalan yang mana hayat simpannya hanya 15 h. Asid borik, asid laktik dan sodium metabisulfit berupaya merencat pertumbuhan bakteria psikrofilik dan pembentukan amin biogenik dalam udang sementara memelihara bilangan mesofilik pada aras yang lebih rendah semasa penyimpanan ais.

Penggunaan kaedah enzim melalui kaedah biosensor dalam bentuk elektrod oksigen dan spektrofotometer menawarkan kaedah alternatif yang baik untuk menentukan kandungan histamina dalam udang. Keputusan ujikaji menunjukkan peningkatan histamina semasa penyimpanan diukur melalui signal elektrod oksigen menggunakan enzim diamine oksidase. Korelasi tinggi melebihi 0.98 setanding dengan kaedah HPLC bagi kesemua sampel dan peratus pengesanan semula kira-kira 100% bagi nilai-nilai histamina di antara 0-200 μg/g dicerap. Sejajar dengan ini, penggunaan spektrofotometer melalui sistem gandingan enzim diamine oksidase dan peroksidase horseradish memberi anggaran separa kuantitatif kandungan histamina dalam udang. Koefisien korelasi melebihi r^2 = 0.99 adalah diperolehi apabila perbandingan dibuat dengan kaedah HPLC. Peratus pengesanan semula histamina dalam sampel yang ditambah piawai adalah juga disekitar 100% bagi kepekatan histamina di antara 0-200 μg/g.

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In loving memory of my late husband, Allahyarham Ahmad Ariffin, whom I dearly missed, who sacrificed a lot and help me in the early part of my work, and wished to see me successful and happy all my life, " May Allah Cucuri Rahmat ke atas Roh mu " .

To my husband, Mohd Razali Sahudin, I am utmost grateful and I highly appreciate you for the confidence, being supportive, understanding and loving all the way and for spending time and much more for driving me to get my live prawn samples at various remote places whenever I needed to. My appreciation is not complete unless I extend it to both my children, Ayong and O'meil for being very understanding and putting up with my "busyness".

Last but not least, my thanks goes to Professor Tan Sri Datuk Dr. Syed Jalaludin Syed Salim for giving me the opportunity for this study and to the Malaysian Government and Universiti Putra Malaysia for granting me the study leave and sponsoring the work.

I certify that an Examination Committee met on 28th March 2001 to conduct the final examination of Fatimah binti Abu Bakar on her Doctor of Philosophy thesis entitled "Microbiological and Biochemical Changes of Freshwater Prawns (*Macrobrachium rosenbergii*) During Storage" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.

AINI IDERIS, Ph.D.
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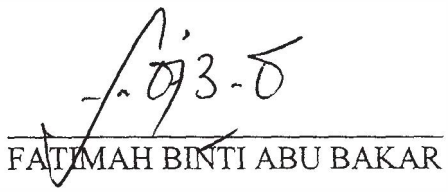
Date:

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or currently for any other degree at UPM or other institutions.



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